

In the Specification:

Replace the initial paper copy of the Sequence Listing with the substitute paper copy of the Sequence Listing filed herewith at the end of the application.

Please amend the paragraph beginning at page 21, line 3 as follows:

For reverse transcription of mRNAs in cells, twenty of the fixed cells were thawed, resuspended in 20 µl of ddH<sub>2</sub>O, heated to 65 °C for 3 min and then cooled on ice. A 50 µl RT reaction was prepared, comprising 5 µl of 10x in-cell RT buffer (1.2M KCl, 0.5M Tris-HCl, 80 mM MgCl<sub>2</sub>, 10 mM dithiothreitol, pH 8.1 at 42 °C), 5 µl of 5 mM dNTPs, 25 pmol oligo(dT)<sub>n</sub>-T7 promoter [(SEQ ID NO. 1)], 80U RNase inhibitor and above cold cells. After reverse transcriptase (40U) was added, the RT reaction was mixed and incubated at 55 °C for three hours. The cells were then washed once with PBS and resuspended in a 50 µl tailing reaction, comprising 2 mM dGTP, 10 µl of 5x tailing buffer (250 mM KCl, 50 mM Tris-HCl, 7.5 mM MgCl<sub>2</sub>, pH 8.3 at 20 °C). The tailing reaction was heated at 94 °C for 3 min and then chilled in ice for mixing with terminal transferase (20U), following further incubation at 37 °C for 20 min. Final reaction was stopped at 94 °C for 3 min. The reaction mixture was chilled in ice immediately, which formed the poly(N)-tailed cDNAs.

Please amend the paragraph beginning at page 22, line 3 as follows:

A 50 µl RT reaction was prepared, comprising 5 µl of 10x RT buffer (300 mM KCl, 0.5M Tris-HCl, 80 mM MgCl<sub>2</sub>, 10 mM dithiothreitol, pH 8.3 at 20 °C), 5 µl of 5 mM dNTPs, 25 pmol oligo(dC)<sub>n</sub>-T7 promoter [(SEQ ID NO. 2)], 80U RNase inhibitor, ddH<sub>2</sub>O and 5 µl of the above aRNA containing supernatant. After reverse transcriptase (40U) was added, the RT reaction was vortexed and incubated at 55 °C for three hours. The resulting products of RT can be directly used in following PCR reaction (50 µl), comprising 5 µl of 10x PCR buffer (Boehringer Mannheim), 5 µl of 2 mM dNTPs, 25 pmol T7-20mer primer, 25 pmol poly(dT)-26mer primer [(SEQ ID NO. 3)], ddH<sub>2</sub>O, 5 µl of above RT product and 3U of Taq/Pwo long-extension DNA

polymerase. The PCR reaction was subjected to thirty cycles of denaturation at 95 °C for 1 min, annealing at 55 °C. for 1 min and extension at 72 °C for 3 min. The quality of final amplified cDNA library (20 µl ) was assessed on a 1% formaldehyde-agarose gel, ranging from 100 bp to above 12 kb.

Please amend the paragraph beginning at page 22, line 17 as follows:

**Pre-cycling procedures.** Primers used in RNA-PCR were as follows: a poly(dT)<sub>24</sub> primer (5'-TTTTTTTTTTTTTTTTTTTTTTTTTTT-3') (SEQ ID NO. [12] 1) and an oligo(dC)<sub>10</sub>N-promoter primer mixture comprising equal amounts of oligo(dC)<sub>10</sub>G-T7 primer (5'-dCCAGTGAAT[-F]TGTAATACGACTCACTATAGGGAAC<sub>10</sub>G-3') (SEQ ID NO. [13] 2); oligo(dC)<sub>10</sub> A-T7 primer (5'-dCCAGTGAAT[-F]TGTAATACGACTCACTATAGGGAAC<sub>10</sub>[T]A-3') (SEQ ID NO. [14] 3); and oligo(dC)<sub>10</sub>T-T7 primer (5'-dCCAGTGAATTGTAATACGACTCACTATAGGGAAC<sub>10</sub>T-3') (SEQ ID NO. [15] 4). The poly(dT)<sub>24</sub> primer was used to reverse transcribe mRNAs into first-strand cDNAs, while the oligo(dC)<sub>10</sub>N-promoter primers functioned as a forward primer for second-strand cDNA extension from the poly(dG) end of the first-strand cDNAs and therefore RNA promoter incorporation. All oligonucleotides were synthetic and purified by high performance liquid chromatography (HPLC).

Please amend the paragraph beginning at page 25, line 5 as follows:

Few fixed and permeabilized cells were applied to a reaction mixture (20 ml) on ice, comprising 2 ml of 10x RT&T buffer (400 mM Tris-HCl, pH 8.3 at 25 °C, 400 mM NaCl, 80 mM MgCl<sub>2</sub>, 5M betaine, 100 mM DTT and 20 mM spermidine), 1 mM Shh-antisense primer [(SEQ ID. NO. 4)], 1 mM Shh-sense promoter-primer [(SEQ ID. NO. 5)], 2mM rNTPs, 2mM dNTPs and RNase inhibitors (10U). After C. *therm.*/Taq DNA polymerase mixture (4U) was added, the reaction was incubated at 52 °C for 3 min, at 65 °C for 30 min, at 94 °C for 3 min, at 52 °C for 3 min, and then at 68 °C for 3 min. A transcription reaction was prepared by adding T7 RNA

polymerase (200U) and *C. therm.* polymerase (6U) mixture into above reaction. After one hour incubation at 37 °C, the resulting mRNA transcripts were continuously reverse-transcribed into mRNA-cDNA duplexes at 52 °C for 3 min, and then at 65 °C for 30 min. The quality of amplified mRNA-cDNA products can be assessed on a 1% formaldehyde-agarose gel (Lin *et al.*, Nucleic Acid Res. (1999)).

Please amend the paragraph beginning at page 26, line 4 as follows:

For  $\beta$ -catenin, a double-stranded DNA template fragment, a pair of primers was designed based on the cDNA sequence. The central region for antisense targeting of  $\beta$ -catenin (aa 306 – 644) required four primers (*i.e.*, primers A-D). The upstream (A) primer comprises the sequence 5'-ATGGCAATCAAGAAAGTAAGC-3' (SEQ ID. NO. [6]5). The downstream (B) primer comprises the sequence 5'-GTACAACAACCTGCACAAATAG-3' (SEQ ID. NO. [7]6). Another set of primers was required for the generation of the desired duplexes. The (C) primer was generated by adding the T7 promoter (RP) before the 5' end of the (A) primer. The (D) primer was generated by adding the T7 promoter before the 5' end of the (B) primer.

Amend the paragraph beginning at page 28, line 9 as follows:

Four synthetic oligonucleotides were used in the generation of bcl-2 RNA-DNA hybrids as follows: T7-bcl2 primer (5'-dAAACGACGGCCAGTGAATTGTAATACGACTCACTATAGGCGGATGACTGAGTACCTGAACCGGC-3') (SEQ ID. NO. [8]7) and anti-bcl2 primer (5'-dCTTCTTCAGGCCAGGGAGGCATGG-3') (SEQ ID. NO. [9]8) for mRNA-cDNA hybrid (D-RNAi) probe preparation; T7-anti-bcl2 primer (5'-dAAACGACGGCCAGTGAATTGTAATACGACTCACTATAGGCCTTCTTCAGGCCAGGGAGGCATGG-3') (SEQ ID NO. [10]9) and bcl2 primer (5'-dGGATGACTGAGTACCTGAACCGGC-3') (SEQ ID NO. [11]10) for antisense RNA (aRNA)-cDNA hybrid (reverse D-RNAi) probe preparation. The design of the sequence-specific primers is based on the same principle used by PCR (50~60% G-C

rich), while that of the promoter-linked primers however requires a higher G-C content (60~65%) working at the same annealing temperature as above sequence-specific primers due to their unmatched promoter regions. For example, new annealing temperature for the sequence-matched region of a promoter-linked primer is equal to  $[2^{\circ}\text{C} \times (\text{dA} + \text{dT}) + 3^{\circ}\text{C} \times (\text{dC} + \text{dG})] \times 5/6$ , not including the promoter region. All primers were purified by polyacrylamide gel electrophoresis (PAGE) before use in RNA-PCR reaction.